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Reference:

Grunst Andrea, Grunst Melissa, Daem Natasha, Pinxten Rianne, Bervoets Lieven, Eens Marcel.- An important personality trait varies with blood and plumage metal concentrations in a free-living songbird
Environmental science and technology / American Chemical Society - ISSN 0013-936X - 53:17(2019), p. 10487-10496
Full text (Publisher's DOI): <https://doi.org/10.1021/ACS.EST.9B03548>
To cite this reference: <https://hdl.handle.net/10067/1628140151162165141>

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Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.9b03548 • Publication Date (Web): 02 Aug 2019

Downloaded from pubs.acs.org on August 6, 2019

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Effect on exploration behavior, an avian personality trait

Metal pollution

An important personality trait varies with blood and plumage metal concentrations in a free-living songbird

Metal exposure and avian personality

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Abstract

Metal pollution is a global problem, which threatens to seriously disrupt behavioral patterns and health in humans and wildlife. Nonetheless, little is known regarding how exposure to metal pollution affects animal personalities, as defined by repeatable among-individual differences in behavior. We used a large dataset to investigate the relationship between individual blood and feather metal concentrations and three personality traits (exploration behavior, territorial aggressiveness, and aggressiveness during nest defense) in great tits (*Parus major*), a model species for animal personality research. We previously demonstrated slower exploration behavior at highly polluted study sites, where exposure to lead, cadmium and arsenic is high. Here, we demonstrate the across-year repeatability of exploration behavior and aggressiveness

during nest defense, providing strong evidence for the existence of personalities in our populations. Furthermore, we demonstrate that individuals with high blood lead concentrations, and high concentrations of multiple metals in the feathers, exhibit slower exploration behavior, but no differences in territorial aggressiveness or nest defense, relative to less exposed birds. The mechanism underlying the relationship between metal exposure and exploration behavior remains to be determined, but could involve neurotoxic effects. Our study highlights that metal pollution could have underappreciated effects on animal personalities, with implications for individual fitness and societal function.

Key words: metal pollution, lead, personality, exploration behavior, aggressiveness, *Parus major*

Introduction

Anthropogenic contaminants threaten the integrity of natural ecosystems, and the health of humans and wildlife in polluted areas. Although policies have been adopted to limit emissions in many countries, exposure continues, with many pollutants persisting in the environment long after emissions have ceased^{1,2}. Metals are persistent inorganic pollutants that can have particularly detrimental biological effects due to their capacity to elevate oxidative stress³, and exert neurotoxic^{4,5} and genotoxic effects^{6,7}. High levels of metal exposure can result in acute poisoning, which can be lethal⁸. At lower concentrations, metal exposure during development or adulthood can also result in sublethal effects on many different aspects of organismal function. Lead (Pb) and other metals can have substantial effects on cognitive^{4,9,10} and behavioral traits¹¹⁻¹⁴ in both humans and wildlife even at very low concentrations. For instance, golden eagles (*Aquila chrysaetos*) with blood Pb levels measured in the parts per billion exhibited reduced activity levels¹³.

Many of the behavioral traits that can be affected by metal exposure are traits that are central to animal personalities. Personalities are defined by repeatable, inter-individual differences in behavioral traits including exploration behavior, aggressiveness, and risk-sensitivity^{15,16}, and are commonly multidimensional, consisting of suites of inter-correlated traits^{15,17}. Personalities frequently have a

genetically-determined component^{18,19}, but are also influenced by environmental effects, potentially including exposure to pollutants^{20,21}. Variation in personality is often thought to be maintained by selective mechanisms²²⁻²⁴. For example, variation in personality could be maintained through frequency-dependent selection²³, or different personalities could function better in different environments²⁴. However, exposure to pollutants could cause non-adaptive variation in personalities. For example, metals including Pb and cadmium (Cd) are neurotoxins that can disrupt neurotransmission, resulting in behavioral dysregulation^{12,25-27}. Given the widespread exposure of humans and wildlife to pollutants, anthropogenic contaminants could play a non-trivial role in determining variation in personalities, with implications for individual fitness and social function²¹.

We investigated variation in personality traits in great tits (*Parus major*), a common European songbird which is a model species for animal personality research. We studied populations that are exposed to varying levels of metal pollution, which is emitted from a non-ferrous smelter south of Antwerp, Belgium. This facility is a major point source for metals, especially Pb, Cd and arsenic (As)²⁸⁻³⁶. In a recent study, we found that an important personality trait, novel environment exploration behavior (hereafter referred to as exploration behavior), is reduced in great tits inhabiting areas close to the metal pollution source³⁵. Exploration behavior is employed as a proxy for personality variation in great tits. Individuals that rapidly explore novel environments are referred to as fast explorers, and also tend to be more aggressive^{17,37,38}, bolder towards novel objects^{19,36}, and less stress responsive^{39,40} than slow explorers. Exploration behavior is repeatable in great tit populations across Europe⁴¹, has a genetically-determined component⁴², and can affect fitness²⁴. In contrast to exploration behavior, two other personality traits, territorial aggressiveness and aggressiveness during nest defense, did not vary across the pollution gradient³⁵. These results suggest that, rather than suites of traits shifting in concert with metal exposure, some personality traits are more sensitive to exposure than others.

However, in our previous study, we only analyzed behavioral variation among study sites, whereas variation in metal exposure can also occur on the individual level within sites⁴³. Thus, in this study, we further examined the potential effect of metal pollution on personality traits by relating individual blood

and feather metal concentrations to the traits considered in our previous study, namely exploration behavior, territorial aggressiveness, and female aggressiveness during nest defense (hissing behavior). Using two years of data, we also assessed the across-year replicability of our results, and the individual-level repeatability of exploration behavior, hissing behavior and metal exposure levels. Our results suggest that exposure to metal pollution could have underappreciated effects on personalities in free-living animals.

Methods

Study system: We studied great tits residing in five urban populations which are located at different distances (0–600 m (site UM), 2500 m (F7), 4000 m (F6), 5000 m (F5) and 8500 m (F4)) from the Umicore smelter and metal refinery in Hoboken (Antwerp), Belgium. The Umicore facility is a major point source for metals, especially Pb and Cd, but also including As and copper (Cu). Metal deposition is high near the smelter, sometimes exceeding emission limits set by Flemish and European authorities, and exponentially decreases with distance^{28-36,44}. Thus, the study populations are exposed to different levels of metal pollution, but variation in metal exposure also occurs on the individual level, within study sites⁴³. All study sites are similar in habitat type and nest box density. Furthermore, movements of great tits between study sites are extremely limited, even during the winter. Indeed, individual birds used in this study were repeatedly captured during the winters of 2017 and 2018 within the same study site, often in the same nest box, and birds caught during the winter at one study site also always breed at this study site. Thus, exposure to pollution is expected to be repeatable within individual birds based on fidelity to sites (and territories) that differ in pollution levels.

This study was approved by the University of Antwerp ethical committee (ID 33480) and performed in accordance with Belgium and Flemish laws. The Belgian Royal Institute for Natural Sciences provided banding licenses for authors and technical personnel.

Measuring metal exposure: We measured metal concentrations in red blood cells (RBCs) sampled in 2017 and 2018, and feathers collected in 2017. Most blood and feather samples were collected immediately following exploration tests, and ~3-4 months before we conducted tests on territorial aggressiveness and nest defense behavior. However, we obtained a small subset of samples from birds for which we measured territorial aggressiveness (21/69 and 12/66 samples from males and females, respectively) and hissing behavior (13/130 samples) during the breeding season. These samples were collected during the nestling period, and thus were not obtained in close coordination with behavioral testing. However, we demonstrate repeatability in individual blood metal levels (see below), and feather metal levels indicate longer-term patterns of metal exposure²⁹. Thus, we believe that it is nonetheless valid to test the relationship between territorial aggressiveness, nest defense behavior, and individual blood and feather metal levels.

We measured RBC and feather concentrations of five metals (As, Cd, Cu, Pb and Zn) using inductively coupled plasma mass spectrometry (7700× ICP-MS, Agilent Technologies, Santa Clara, CA, USA), using standard methods^{29-31,43,45-48}. Methodological details can be found in the Supporting Material. Blood metal concentrations were calculated in µg/g on a wet weight basis, whereas feather metal concentrations were calculated in µg/g on a dry weight basis. RBC metal measurements represent exposure levels over a slightly longer timeframe (several months) than plasma measurements⁴⁹. There may also be some differences as to which metals are primarily absorbed by the RBCs versus plasma⁴⁹. Nonetheless, metal levels in the whole blood and RBCs are expected to be correlated⁵⁰. We have done extensive past work in great tits and other species which demonstrates that blood and feather measurements can be used as reliable indicators of metal exposure levels, and are related to metal levels in internal organs^{28-31,46}. However, we acknowledge that feather metal measurements are subject to external contamination, and might thus less accurately reflect internal exposure levels than blood metal concentrations⁵¹.

Behavioral assays

Exploration test: We performed exploration tests during the winters of 2017 and 2018. Data from 249 birds tested in 2017 are published in our previous study on the same populations³⁵. Our current dataset incorporates a second year of data (2018). Thus, we now have 629 observations on 498 birds (380 additional tests, 249 additional birds). This large dataset allows us to test the across-year repeatability of exploration behavior and the replicability of our previous results. Moreover, our previous study did not include data on individual metal exposure levels. These data are critical to establishing a connection between metal exposure and behavioral patterns on the individual level. Sample sizes were reduced for analyses regarding blood and feather metal levels, so we report all sample sizes in the results section.

To perform the exploration test, we captured birds sleeping in nest boxes between December 4 and March 2, 2017 and January 15 and March 7, 2018. We transported birds to the University of Antwerp, where they were housed overnight in individual cages. The following morning, between 8:15 and 10:00, we used standardized protocol to perform the exploration test^{35,38,41,42}. Briefly, we observed the movement of each bird in the novel environment room for two minutes, and noted the numbers of hops and flights. Exploration scores were calculated as the total number of hops and flights within 2 min. This measurement quantifies the speed of early exploration behavior upon an individual's entry into the novel environment, is used in laboratories across Europe⁴¹, and is proportional to the time it takes for birds to visit all areas of the test room⁴².

Immediately after testing all birds, we sexed and aged (yearling or older) birds based on plumage characteristics⁵². We gave all birds a metal band and color band combination, and fitted females with passive integrated transponder (PIT) tags (IB Technology, Aylesbury, UK). Finally, we obtained ~150 μ L blood samples and plucked the second to outermost left rectrix (in 2017) to assay metals. We then released birds near the nest boxes where they were captured.

Aggression during nest defense (hissing test): When confronted by a predator entering the nest cavity, female great tits perform a hissing display, consisting of producing loud vocalizations and shaking the wings⁵³⁻⁵⁵. We quantified this behavior during the breeding seasons of 2017 and 2018 by inserting the

head of a taxidermic mount of a great spotted woodpecker (*Dendrocopos major*) into the hole of the nest box and counting the number of vocalizations produced within one minute. Four different decoys were used. Following the one-minute trial, we opened the nest box to confirm the presence of the female. The identity of PIT-tagged females was verified by scanning the nest box with a handheld transponder reader (GR-250 RFID Reader, Trovan, Aalten, Netherlands).

Hissing tests were performed by one of ten observers. Data from 273 tests on 152 females, which were performed in 2017, are already published³⁵. Our current dataset includes 719 tests on 361 females (446 additional tests, 209 additional females) across two years, which allows us to test the across-year repeatability of behavior and the robustness of previous results. Moreover, hissing data were not previously analyzed with respect to individual metal exposure levels. In 2017, we performed tests between March 28 and April 26, between 8:00 and 17:00. In 2018, tests were performed between April 17 and May 2, between 8:00 and 18:00. Tests were performed during the incubation stage (day 1 to 12; mean $\pm$ SE = 5.452 $\pm$ 0.126) of first clutches, with most females tested multiple times across the incubation stage.

Territorial aggression (Simulated territory intrusions, STIs): We performed STIs during the breeding season of 2017. Data from these tests and methodological details are already published³⁵, but were not analyzed with respect to individual metal exposure levels. For analyses using blood metal measurements, sample sizes were 103 tests on 66 birds for males, and 90 observations on 57 birds for females. For analyses using feather metal measurements, sample sizes were 70 tests on 47 males, and 77 tests on 47 females. STIs were performed by placing a taxidermic mount of an adult male great tit and a song recording on top of the nest box and recording the response of the territory owner for three minutes (approach distance, time spent on the decoy, and the number of alarm calls, songs, dives, and attacks on the decoy). Our STIs focused on males, which are easily distinguished from females based on the larger size of the black, melanin-based breast stripe⁵². However, when the female was present during the STI, we also quantified her response to the intruder. We recorded her approach distance, and scored her

aggressive response on a scale of 0-3, with 0 corresponding to sitting near the nest box, 1 to alarm calling, 2 to sitting on the decoy, and 3 to attacking the decoy. We used principal component analysis to collapse variables describing male aggressiveness into a few orthogonal variables (PC1 and PC2). PC1 explained 32.8% of the variance in behavior, and separated non-aggressive males that sang at a distance from the decoy from aggressive males that approached and attacked the decoy. PC2 explained 27.4% of the variance, and characterized the tradeoff between calling and diving at the decoy versus directly attacking³⁵. Here we only investigated the relationship between male aggressiveness PC1 and metal exposure, because only PC1 exhibited individual repeatability, and can thus be considered a personality trait³⁵. Female territorial aggressiveness was described using a single principal component (PC1)³⁵. STIs were not performed at F6 due to logistical constraints.

Statistical analyses

Variation in metal exposure: We performed all statistical analyses in R 3.4.0⁵⁶. We used linear mixed effects models (LMM, R package lme4)⁵⁷ to assess how blood metal concentrations (log-transformed) varied among study sites, and with sex, capture date, and age (fixed effects). We entered individual identity and year as random effects, and estimated *P*-values using Satterthwaite approximations for degrees of freedom (R package lmerTest)⁵⁸. For feather metals, we did not have repeated measures on individuals across years, so performed an equivalent analysis using a linear model. Concentrations of different metals measured in feathers were highly correlated, whereas correlations between blood metal levels were relatively low (see results). Thus, before investigating relationships between feather metal concentrations and behaviors, we used principal components analysis (R function prcomp) to compress feather metal concentrations into a few orthogonal variables (PC1 and PC2). Post-hoc comparisons among sites utilized the Tukey method.

Behavioral variation with metal exposure levels: To explore the association between individual metal exposure and behaviors, we constructed two separate models for each behavior, one using blood metal

concentrations (Pb, Cu, Zn) and the other using feather metal concentrations (PC1 and PC2). For exploration and hissing behavior, we used generalized linear mixed effects models (GLMMs) with a Poisson distribution. In global models, we entered the metal concentration variables, age, date, and test number (i.e. first versus second test on an individual, counted across years for exploration behavior but within years for hissing behavior) as fixed effects, and individual identity, study site, and year as random effects. We added an observation-level random effect to control for over-dispersion⁵⁹. We used sex as an additional fixed effect for the model involving exploration behavior. The model involving hissing behavior used time of day as an additional fixed effect, and decoy identity as an additional random effect. Feather metal concentrations were measured only in 2017. Thus, models using feather metal PCs did not include year as a random effect (nor individual, for the exploration behavior model), but were otherwise identical to models using blood metal concentrations.

For territorial aggressiveness in males and females, we used LMMs to predict the first principal component (PC1) describing variation in metrics of aggressiveness from blood metal concentrations and feather metal PCs (in separate models), age, date, and test number (fixed effects). Individual identity, study site, decoy number, and recording number were used as random effects.

We also analyzed variation in personality traits among study sites. This analysis replicates our previous study³⁵ on personality traits in these study sites using an additional year of data. Furthermore, we used a Poisson GLMM to assess whether a relationship existed between hissing and exploration behavior across the two years. We entered hissing behavior as the dependent variable and exploration behavior (in the same year) as the predictor, and used the same additional predictors as above. For the site-level analysis, we did not include female age in the model for hissing behavior, because 119 females tested for hissing (but not blood or feather sampled) were not aged, and our past³⁵ and current analyses indicate no effect of age on hissing behavior.

For the models involving the relationship between individual metal levels and behavioral traits we used model selection based on AICc, followed by model averaging of the best-supported models ($\Delta AICc < 2$) to calculate model-averaged coefficient estimates with 95% confidence intervals and assess the

importance of fixed effect predictors (R package MuMIn)⁶⁰. For other models, inferences were based on global models. According to Burnham and Anderson⁶¹, models with $\Delta\text{AICc} < 2$ are the most highly supported, models with ΔAICc from 4-6 have substantially less support but cannot be discounted, and models with $\Delta\text{AICc} < 10$ are highly unlikely. We included only models with $\Delta\text{AICc} < 2$ in model averages. However, results were qualitatively equivalent when averaging all models with $\Delta\text{AICc} < 7$, and we present all models with $\Delta\text{AICc} < 7$ in the Online Supplement for comparative purposes.

All predictor variables were standardized to a mean of zero and a standard deviation of one. Thus, we present standardized beta coefficients (mean $\pm$ SE), which give a sense of relative effect sizes. In addition, we also present marginal R^2 values for models with $\Delta\text{AICc} < 2$, null models, global models, and best-supported models excluding the metal variables (which represent alternative null models). R^2 values were calculated following Nakagawa and Schielzeth⁶² (function `r.squaredGLMM` in package MuMIn), and describe the proportion of the total variance explained by fixed effects⁶².

Repeatability in metal exposure and behaviors: We used R package rptR⁶³ to estimate individual repeatability in blood metal concentrations, exploration behavior and hissing behavior based on variance components from mixed effects models. To maximize sample sizes for the behavioral data, we used models containing only study site, sex, age (for exploration behavior), date, and time (for hissing). Bootstrapping was used to derive 95% confidence intervals, and P -values were obtained using log-likelihood ratio tests. When analyzing the repeatability of behavioral traits, we used only the first observation taken on each individual within each year.

Results

Metal exposure levels: Lead, Cu, and Zn were present in the blood at detectable levels (Fig. 1a-c), whereas Cd and As were below the detection limit (levels < 0.05 $\mu\text{g/g}$) in almost all samples. Lead, Cu, Zn, Cd, and As were above the detection limit in the feathers (Fig. 1d-h). Correlations between metal levels measured in the blood were relatively moderate ($r_{\text{Pb,Cu}} = 0.16$, $r_{\text{Pb,Zn}} = 0.17$, $r_{\text{Cu,Zn}} = 0.14$, $P < 0.01$,

$N = 427$), whereas correlations between metal levels measured in feathers were comparatively high ($r_{\text{Pb,As}} = 0.79$, $r_{\text{Pb,Cd}} = 0.82$, $r_{\text{Pb,Cu}} = 0.77$, $r_{\text{Pb,Zn}} = 0.37$, $r_{\text{As,Cd}} = 0.77$, $r_{\text{As,Cu}} = 0.63$, $r_{\text{As,Zn}} = 0.17$, $r_{\text{Cd,Cu}} = 0.71$, $r_{\text{Cd,Zn}} = 0.28$, $r_{\text{Cu,Zn}} = 0.44$, $P < 0.01$, $N = 250$). We found no effect of sex, age or sampling date on metal concentrations in the blood or feathers ($P > 0.10$). Blood Pb concentrations ($F_{4,320} = 80.952$, $P < 0.001$), and feather Pb, Cd, As and Cu concentrations were higher at site UM relative to at other sites ($P < 0.001$), but also varied substantially within sites (Fig. 1). In our PCA, PC1 explained 77.3% of the variance in feather metal levels, was higher at UM than at other sites ($P < 0.001$), and had high positive loadings for Pb (0.501), Cd (0.489), Cu (0.492), and As (0.476), and a lower loading for Zn (0.198). PC2 explained 17.9% of the variance, did not vary between sites ($P > 0.05$), and had a high positive loading for Zn (0.971), and low negative loadings for Pb (-0.094), Cd (-0.029), Cu (-0.065), and As (-0.206).

Figure 1. Boxplots showing metal concentrations in the (a-c) blood and (d-h) feathers of great tits from study sites at different distances from the metal pollution source. Whiskers extend from the first and third quartiles to the highest value within 1.5 times the interquartile range. Distance from the pollution source increases from site UM to site F4, as moving right along the x-axis. Blood metal concentrations were measured in $\mu\text{g/g}$ wet weight and feather metal concentrations in $\mu\text{g/g}$ dry weight. Note that the y-axis is on a log scale.

Behavioral variation with individual levels of metal exposure

Exploration behavior: We found support for a negative relationship between blood Pb concentrations and exploration behavior. Blood Pb appeared in all models with $\Delta\text{AICc} < 2$. Model averaging indicated that blood Pb, test number and date contributed substantially to explaining variation in exploration behavior, with birds that had higher blood Pb levels exhibiting slower exploration behavior (Table 1a; $N=412$ observations, 337 birds; see Supplementary Table S1 for models with $\Delta\text{AICc} < 7$). Blood Cu also appeared in the top model, but the 95% interval for the model-averaged coefficient estimate was non-significant ($P = 0.234$), with a 95% CI overlapping zero (Table 1a). A negative relationship between blood Pb and exploration scores was also supported by model-averaged coefficient estimates within 2017 ($\beta = -0.518 \pm 0.147$ (-0.809, -0.226), $Z = 3.485$, $P = <0.001$, $N = 228$) and 2018 ($\beta = -0.247 \pm 0.126$ (-0.496, 0.001), $Z = 1.950$, $P = 0.051$, $N = 184$). Moreover, the relationship was supported by the model-averaged coefficient estimate for blood Pb concentrations within the most polluted UM study site ($\beta = -0.368 \pm 0.164$ (-0.710, -0.053), $Z = 2.281$, $P = 0.022$).

Model averaging also suggested a negative relationship between feather metal PC1 and exploration behavior, whereas feather metal PC2 was not included in any of three models with $\Delta\text{AICc} < 2$ (Table 1b; see Supplementary Table S2 for all models with $\Delta\text{AICc} < 7$). The negative relationship between feather metals and exploration behavior also held for the metals that showed high positive loadings on PC1, namely Pb (Fig. 2b), Cd, As and Cu ($P < 0.01$).

Exploration behavior also varied among study sites ($\chi^2_4 = 10.404$, $P = 0.034$), with birds at the polluted UM site having the slowest exploration behavior and birds at F5 having the fastest exploration behavior (Supplementary Table S3). However, among site differences were weaker across the two years of our study than within 2017, because there were no statistically significant differences in exploration behavior among study sites in 2018 (Supplementary Fig. S1).

Table 1. GLMMs with $\Delta\text{AICc} < 2$ and model-averaged coefficient estimates for fixed effects predicting the exploration behavior of great tits from (a) blood metal concentrations and (b) feather metal concentrations. The null model, global model and the best-supported model with blood Pb, or feather metal PC1, eliminated are shown for comparison. Marginal R^2 values give measures of effect sizes.

a) Blood metal models ^a						b) Feather metal models ^b					
Models	K	AICc	ΔAICc	Weight	R^2	Model	K	AICc	ΔAICc	Weight	R^2
Pb + Cu + D + TN	9	2957.22	0	0.40	0.126	PC1 + D + TN	6	1802.62	0	0.43	0.080
Pb + D + TN	8	2958.34	1.12	0.23	0.118	PC1 + D	5	1802.72	0.10	0.41	0.070
Pb + Cu + D + TN + S	10	2958.81	1.58	0.18	0.127	PC1 + D + TN + S	7	1804.48	1.87	0.17	0.081
Pb + Cu + D + TN + A	10	2958.81	1.59	0.18	0.127	Global model	9	1808.75	6.13		0.081
Global model	12	2962.48	5.26		0.128	w/o PC1 (D + TN)	5	1809.20	6.58		0.038
w/o Pb (Cu + D + TN)	8	2967.03	9.81		0.083	Null model	3	1813.62	11.00		0
Null model	5	2996.71	39.49		0						
Model-averaged coefficients						Model-averaged coefficients					
Fixed effects	$\beta \pm \text{SE}$	95% CI	Z	P		Fixed effects	$\beta \pm \text{SE}$	95% CI	Z	P	
Intercept	1.748 $\pm$ 0.095	1.560, 1.936	18.287	< 0.001		Intercept	2.265 $\pm$ 0.194	1.885, 2.645	16.975	< 0.001	
Blood Pb	-0.319 $\pm$ 0.096	-0.514, -0.123	3.202	0.001		PC1	-0.151 $\pm$ 0.050	-0.250, -0.052	2.998	0.002	
Blood Cu	0.114 $\pm$ 0.099	-0.014, 0.313	1.189	0.234		Date	-0.829 $\pm$ 0.290	-1.399, -0.259	2.851	0.004	
Date	-0.365 $\pm$ 0.075	-0.513, -0.217	4.845	< 0.001		Test num.	0.096 $\pm$ 0.115	-0.050, 0.375	0.831	0.404	
Test num.	0.277 $\pm$ 0.076	0.127, 0.427	3.631	< 0.001		Sex	-0.015 $\pm$ 0.083	-0.456, 0.271	0.185	0.852	
Age	-0.022 $\pm$ 0.087	-0.460, 0.215	0.255	0.798							
Sex	-0.020 $\pm$ 0.080	-0.421, 0.195	0.257	0.796							
Random effects	Variance	SD	N			Random effects	Variance	SD	N		
Observation	1.355	1.164	412			Observation	1.788	1.337	249		
Individual	0.549	0.741	337			Study site	<0.001	<0.001	5		
Study site	<0.001	<0.001	5								
Year	<0.001	<0.001	2								

Pb = blood Pb, Cu = Blood Cu, Zn = Blood Zn, D = date, TN = test number, S = sex, A = age, PC1 = feather metal PC1 score, PC2 = feather metal PC2 score

^aN (observations, birds) = total: 412, 337; F4: 56, 47; F5: 50, 43; F6: 94, 73; F7: 104, 86; UM: 108, 88.

^bN (birds) = total: 249; F4: 32; F5: 26; F6: 81; F7: 48; UM: 62.

Figure 2. Negative relationship between the speed of exploration behavior in great tits and individual concentrations of lead (Pb) in the (a) blood and (b) feathers. Blood Pb concentrations were measured in µg/g wet weight and feather metal concentrations in µg/g dry weight. Darker points are produced by overlapping data points, indicating more data in these regions.

Aggressiveness during nest defense (hissing behavior): We found no evidence that female hissing behavior is affected by metal exposure. Of six models with $\Delta\text{AICc} < 2$, none contained blood Pb or Cu concentrations (Supplementary Table S4 contains all models with $\Delta\text{AICc} < 7$). Only two of these models contained blood Zn, and the model-averaged coefficient estimate for Zn was non-significant, with a 95% CI overlapping zero ($\beta = 0.017 \pm 0.050$ (-0.105, 0.217), $Z = 0.337$, $P = 0.736$) (Supplementary Table S4; $N = 204$ observations, 112 females). For feather metals, of nine models with $\Delta\text{AICc} < 2$, some contained feather metal PC1 and PC2 (Supplementary Table S5 contains all models with $\Delta\text{AICc} < 7$). However, the model-averaged coefficient estimates for both PC1 ($\beta = -0.102 \pm 0.104$ (-0.335, 0.027), $Z = 0.979$, $P = 0.328$) and PC2 ($\beta = 0.024 \pm 0.077$ (-0.120, 0.403), $Z = 0.319$, $P = 0.750$) were non-significant (Supplementary Table S5; $N = 145$ observations, 78 females).

Our site-level analysis indicated that hissing behavior differed among study sites across the two years of our study ($\chi^2 = 32.231$, $P < 0.001$, $N = 719$ observations, 360 females). Consistent with our previous analysis³⁵, females hissed more at F6 than at other sites ($P < 0.01$ in all cases; Supplementary Table S6), rather than behavior differing between the polluted UM site and other sites. Female hissing and

exploration behavior were not correlated ($\beta = -0.028 \pm 0.082$, $Z = -0.343$, $P = 0.731$, $N = 348$ observations, 166 females).

Territorial aggressiveness: Male territorial aggressiveness (PC1) was not related to blood metal concentrations. Only blood Pb (not blood Cu or Zn) appeared in the models with $\Delta\text{AICc} < 2$ (see Supplementary Table S7 for all models with $\Delta\text{AICc} < 7$), and the model-averaged coefficient estimate for blood Pb was non-significant ($\beta = -0.024 \pm 0.082$ (-0.454, 0.128), $Z = 0.301$, $P = 0.763$, $N = 103$ observations, 63 males). Male territorial aggressiveness was also unrelated to feather metal concentrations. Neither feather metal PC1 nor PC2 appeared in four models with $\Delta\text{AICc} < 2$, and the null model was the fourth best model in the candidate model set (Supplementary Table S8 contains all models with $\Delta\text{AICc} < 7$; $N = 70$ observations, 47 males).

Similarly, female territorial aggressiveness was not associated with individual levels of metal exposure. Blood Cu and Zn appeared in some of the models with $\Delta\text{AICc} < 2$, but model-averaged coefficient estimates were non-significant for both Cu ($\beta = -0.152 \pm 0.166$ (-0.534, 0.037), $Z = 0.919$, $P = 0.358$) and Zn ($\beta = -0.007 \pm 0.047$ (-0.411, 0.167), $Z = 165$, $P = 0.869$, $N = 90$ observations, 57 females). Blood Pb did not appear in any of the models with $\Delta\text{AICc} < 2$, and the null model was the fourth best model in the candidate model set (see Supplementary Table S9 for all models with $\Delta\text{AICc} < 7$). Feather metal PC1 and PC2 were also unrelated to female territorial aggressiveness. Feather metal PC1 and PC2 and did not appear in the models with $\Delta\text{AICc} < 2$, and the null model was the best-supported model (Supplementary Table S10 contains all models with $\Delta\text{AICc} < 7$; $N = 77$ observations, 47 females).

Repeatability in behavior and blood metal concentrations: The exploration ($r = 0.416 \pm 0.080$ [0.244, 0.559], $P < 0.001$; $N = 629$ observations, 498 birds) and hissing ($r = 0.848 \pm 0.047$ [0.746, 0.932], $P < 0.001$, $N = 394$ observations, 358 females) behavior of individual great tits were repeatable across years. Moreover, across two years, individual blood concentrations of Pb ($r = 0.456 \pm 0.087$ [0.28, 0.624], $P <$

0.001) and Zn ($r = 0.258 \pm 0.091$ [0.076, 0.431], $P < 0.001$) were repeatable. On the other hand, blood Cu ($r = 0.019 \pm 0.076$ [0, 0.264], $P = 0.441$) concentrations were not repeatable. When the repeatability estimate for blood Pb was adjusted for the effect of study site, the estimate became lower and non-significant ($r = 0.144 \pm 0.109$ [0, 0.419], $P = 0.107$). Thus, repeatability in blood Pb is largely due to the site fidelity of birds. However, there was also significant repeatability in blood Pb levels within sites UM ($r = 0.388 \pm 0.176$ [0, 0.708], $P = 0.025$) and F6 ($r = 0.472 \pm 0.171$ [0.065, 0.746], $P = 0.007$), where blood Pb levels were highest (see Table 1 for sample sizes). Adjusting for study site had little effect on the repeatability estimate for blood Zn ($r = 0.155 \pm 0.082$ [0, 0.318], $P = 0.012$), indicating that repeatable individual differences in blood Zn are not a function of which study site a bird inhabits.

Discussion

Metal pollution continues to be a worldwide problem. Exposure to metals is well-known to have both lethal and sublethal effects on organisms, including substantial behavioral effects^{4,5,9,12,13}. However, few studies have explored how metal pollution affects animal personalities^{15,16}. In a previous study, we found that exploration behavior was slower in great tits inhabiting areas close to the Umicore metal pollution source³⁵, but did not have individual levels of metal exposure available. Here, we use an additional year of data to demonstrate the across-year repeatability of personality traits, thus providing evidence for the stability of personalities in our population. We also show that blood Pb and Zn levels are repeatable within individuals across years, such that differences in metal exposure levels have the potential to contribute to across-year differences in personalities. Moreover, we demonstrate that individual blood and feather metal concentrations are negatively correlated with the speed of exploration. These new results support the hypothesis that metal exposure might have direct effects on this important personality trait, potentially through neurotoxic effects⁶⁴⁻⁷¹. However, indirect effects of metal pollution on ecological conditions could also play a role in explaining results⁷².

Our current analyses suggest that Pb is the most prevalent metal in the blood of great tits exposed to pollution from Umicore. We found average blood Pb concentrations in adult great tits from site UM of $0.785 \pm 0.197 \mu\text{g/g}$ wet weight in 2017 and $0.894 \pm 0.247 \mu\text{g/g}$ wet weight in 2018. In contrast, Cd and As were below the detection limit in the blood. However, Cd, As and Cu were nonetheless relatively highly elevated in the feathers of birds residing close to the pollution source (Fig. 1). There is evidence from great tits that metal concentrations in tail feathers correlate significantly with concentrations in different internal tissues⁴⁶. Nonetheless, feather metal levels might reflect external contamination more than blood levels⁵¹. Indeed, as noted above, some of the metals that were elevated in the feathers of birds residing near Umicore (e.g. Cd and As) were below the detection limit in blood, and levels of different metals in the blood were not highly intercorrelated, despite large intercorrelations between levels of different metals in feathers. Thus, different metals might be incorporated into blood and other internal systems at different rates, such that levels in feathers might not accurately reflect internal exposure levels. Therefore, of the metals measured, Pb seems most likely to directly affect the physiology and behavior of birds, but other metals present in the mixture of metals emitted by Umicore, and detectable in the feathers, could also have some effect.

To assess whether metal exposure affects personalities, it is important to establish that personalities exist in the population by demonstrating individual repeatability in behavior. Here, we show that exploration and hissing behavior are repeatable across years, with repeatability estimates being relatively high for behavioral traits ($r = 0.417$ and 0.837 , respectively). We thus provide strong evidence for the across-year stability of personalities. Moreover, blood Pb and Zn concentrations were also repeatable within individuals across years. Repeatable differences in blood Pb concentrations were largely attributable to the fidelity of birds to study sites that differed in pollution levels. In contrast, the repeatability of Zn concentrations appeared unrelated to the distribution of birds among sites, but might be attributable to other differences in the territory characteristics of individuals. Repeatable individual differences in metal exposure could contribute to repeatable differences in behavior, thereby contributing to the expression of personalities, especially in the case of Pb exposure. To our knowledge, the finding of

individual repeatability in contaminant levels is relatively novel. This finding could have implications not only for understanding variation in personalities, but also for explaining individual differences in life history trajectories and fitness within polluted landscapes.

Individual levels of metal exposure were associated with exploration behavior in male and female great tits, suggesting an effect of metal exposure on this important personality trait in both sexes. Blood Pb concentrations were negatively correlated with the speed of exploration within both 2017 and 2018. Moreover, there was also a negative correlation between blood Pb concentrations and exploration scores within site UM, where Pb levels are highest. These results suggest that Pb could directly affect exploration behavior. On the other hand, feather metal PC1, which is associated with high concentrations of multiple metals, especially Pb, Cd, Cu, and As, was also negatively correlated with the speed of exploration. As previously noted, feather metal levels might less accurately reflect internal levels of metal exposure than blood metal levels⁵¹, but there is evidence for a correlation between feather metal levels and internal levels of metal exposure in great tits⁴⁶. Thus, the correlation between feather metal levels and exploration behavior suggests a potential effect of exposure to multiple metals on this personality trait, which could involve either direct effects of metals, or indirect effects mediated through changes in ecological conditions^{32,72}.

Direct effects of metals on exploration behavior could be mediated through neurotoxic effects. Lead in particular has well-documented neurotoxic effects that can result in cognitive deficits^{9,10} and changes in movement patterns and exploration behavior⁶⁵⁻⁶⁷. Lead readily crosses the blood brain barrier and can trigger neuronal apoptosis and seriously interfere with neurotransmitter systems, with extensive behavioral implications⁶⁸⁻⁷¹. Disruption of dopaminergic and serotonergic neurotransmission systems might have particular relevance for understanding effects of Pb on exploration behavior^{64,69}. Indeed, exploration behavior in great tits has been linked to polymorphisms at the dopamine and serotonin receptors, suggesting a role for these neurotransmitter systems in regulating exploration behavior⁷³.

Why metal exposure would not also be associated with changes in territorial aggressiveness and nest defense behavior is unclear, since neurotoxic effects of metals, or effects on the endocrine system, could

also be expected to affect these behaviors^{74,75}. However, difference in the neurophysiological and hormonal control of exploration behavior, versus territorial aggressiveness and nest defense, could explain why the first is affected by metal pollution in our study system, whereas the latter are not. In addition, blood samples were primarily obtained during winter. Therefore, it remains possible that we might detect a relationship between aggressive behaviors and blood metal concentrations if blood were sampled during the breeding season. However, the repeatability of blood metal concentrations argues against this contingency.

An alternative hypothesis to explain the relationship between exploration behavior and blood Pb concentrations is that low activity levels during the exploration test reflect ataxia due to acute lead poisoning. In some studies, evidence of lead poisoning has been found at levels equivalent to or lower than our highest blood Pb levels⁷⁶. For instance, cinereous vultures (*Aegypius monachus*) with blood Pb levels averaging 1.571 ± 0.510 $\mu\text{g/g}$ showed evidence of lead intoxication⁷⁶, whereas our highest blood Pb concentration was over 10 $\mu\text{g/g}$. In addition, the relationship between blood Pb concentrations and exploration behavior was in large part driven by low exploration scores among birds with exceptionally high blood Pb (over ~ 3 $\mu\text{g/g}$; Fig. 2a). This pattern lends some credence to the hypothesis that these birds could have been exhibiting acute lead poisoning, and calls the hypothesis that Pb has behavioral effects at lower concentrations into question. However, there was also a relationship between feather metal concentrations and exploration behavior, which was less strongly linked to behavioral patterns among birds with extremely high exposure levels. Moreover, all birds rigorously flew away upon release, and did not exhibit signs of sickness during handling and measurement, suggesting that birds were not suffering from acute poisoning.

Changes in animal personalities as a result of exposure to pollutants could have implications for individual fitness and population dynamics. In the context of our system, slower exploration behavior in great tits exposed to metals could have fitness consequences, especially if reduced movement during the exploration test reflects not a different method of assessing a novel environment, as typically assumed in healthy individuals²³, but a decline in activity related to neurological impairments⁶⁵⁻⁶⁷. Indeed, past

studies have shown that exploration behavior, measured in the laboratory, can predict foraging patterns^{77,78} and fitness in free-living birds²⁴.

Despite remediation efforts, metal pollution persists as a worldwide threat to natural ecosystems and the health of humans and wildlife. As in great tits, important personality traits in other species could be affected by exposure to metal pollution. Thus, determining which personality traits are most sensitive to metal pollution, elucidating mechanisms, and examining effects on animal social systems and population dynamics are important areas for future research.

Acknowledgements

We sincerely thank Peter Scheys for providing support during fieldwork and aiding in collecting blood samples. We thank Geert Eens for logistical support, and Emma Van den Camp, Deborah Molitor, Christina Emonds and Cas Jorissen for helping with behavioral tests. A special thanks to Bert Thys for sharing hissing data from F6 and providing advice on analysing personality traits. We are grateful to Steven Joosen for conducting the metal measurements, and to the museum of the Royal Belgian Institute of Natural Sciences for providing taxidermic woodpeckers. This study was made possible through financial support from the University of Antwerp and FWO Flanders (to ASG: grant IDs: 1.2I35.17N and 1526018N, to MLG: grant ID: 1528018N).

Supporting Information

The online supporting for this manuscript contains: 1) Methods for measure metal concentrations, 2) Full results from model selection and averaging (all models with $\Delta AICc < 7$), 3) Site-level analyses for exploration and nest defense behavior.

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